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3-{2-[Bis-(4-fluorophenyl)methoxy]ethyl}-6-substituted-3,6-diazabicyclo[3.1.1]heptanes as novel potent dopamine uptake inhibitors

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Abstract—A series of analogues **2a**–**i** related to 3-{2-[bis-(4-fluorophenyl)methoxy]ethyl}-8-(1*H*-indol-2-ylmethyl)-3,8-diazabicyclo[3.2.1]octane (**1**) in which the 3,8-diazabicyclo[3.2.1]octane core was replaced by 3,6-diazabicyclo[3.1.1]heptane ring system has been synthesized and evaluated for their ability to inhibit DA reuptake into striatal nerve endings (synaptosomes). Biological data showed that compound **2a**, the closest analogue of lead **1**, possessed an increased reuptake inhibition activity over **1** (**2a**, K_i = 5.5 nM). Replacement of the indole ring with bioisosteric aromatic rings—benzothiophene (**2b**), benzofurane (**2c**), or indene (**2d**)—resulted, with the exception of **2d**, in a double digit nanomolar activity. Changing the indenyl moiety of **2d** with simplified aryl groups led to compounds **2e**–**h** which displayed a similar or slightly decreased activity with respect to the ground term. Naphthalene derivative (**2i**) demonstrated a weaker activity than aromatic analogues.

1. Introduction

(−)-Cocaine is the chief component of *Erythroxylum coca* leaves.[1] Cocaine addiction is a chronic relapsing brain disorder characterized by neurobiological changes leading to the illicit use of cocaine which secondly correlates with exacerbation of the spread of AIDS, hepatitis B and C, and drug resistant tuberculosis.[2]

Cocaine acts as an indirect dopamine agonist by blocking the dopamine transporter in the pleasure/reward center of the brain[3] and this blockade results in an excess of dopamine in the synapses. The resulting increase in synaptic dopamine levels produce an amplification of pleasure sensation such as euphoria, psychostimulation, and other rewarding experiences that can lead to drug addiction.[4]

Unlike the situation with other drugs of abuse, there are currently no safe and effective pharmacotherapies available to treat cocaine abuse.

From the pharmacological perspective, two approaches of medications to aid cessation in the cocaine use have been pursued.

One approach is the so-called cocaine replacement therapy (CRT) or maintenance pharmacotherapy which provides a medication that would have pharmacological similarity to cocaine with the purpose to reduce the cocaine-withdrawal symptoms and the craving for cocaine use. For a replacement therapy to be beneficial to treat cocaine dependence, however, it should be less medically deleterious than the drug of abuse that it is replacing. This goal may be obtained using agents with a proper pharmacokinetic profile that surrogate the supply of low and constant levels of cocaine.

The second approach is that to accomplish the pharmacological antagonism by means of a dopamine receptor

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antagonist or partial agonist with the purpose to reduce rewarding effect of cocaine use.

From a preclinical point of view two major classes of therapeutic agents have been studied such as (1) dopaminergic agents and (2) antidepressants. Other category of compounds such as calcium channel blockers and immune response inducers have also been examined as potential cocaine abuse therapeutic agents.[5,6]

For all of these reasons, immediate therapies with non-addictive and nonabusable agents are needed for the treatment of cocaine abuse worldwide.

In the pursuit of possible medications we have focused our attention on DA uptake blockers having original chemical scaffolds in the belief that novel molecular architectures may lead to desired insights into DAT inhibitor activity.

Recently, K.C. Rice and co-workers[7] have introduced the 3,8-diazabicyclo[3.2.1]octane system as new scaffold in DAT inhibitor field (area) embedded in the structure of 3-{2-[bis-(4-fluorophenyl)methoxy]ethyl}-8-(1*H*-indol-2-ylmethyl)-3,8-diazabicyclo[3.2.1]octane (**1**)[7] (Chart 1) which manifests considerable DAT affinity (IC_{50} = 1.4 ± 0.1 nM) and high uptake inhibition potency (IC_{50} = 40 ± 7 nM).

The first objective of the present study was to replace the 3,8-diazabicyclo[3.2.1]octane template of **1** still sufficiently flexible to permit a variety of conformations with a more rigid 3,6-diazabicyclo[3.1.1]heptane scaffold. This backbone-rigidification would be expected to influence bioactivity profoundly. A second objective of this study was to seek correlations between steric properties of N_6 pendant groups and DAT reuptake inhibitory activity.[8,9]

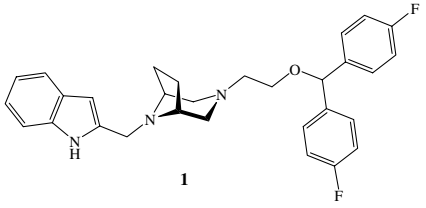
In this context, we chose to synthesize the 3,6-diazabicyclo[3.1.1]heptane analogues of **1** (**2a**–**i**) bearing different groups on the N_6 amine nitrogen with the 2-[bis-(4-fluorophenyl)methoxy]ethyl group at N_3 and to evaluate their DA transporter activity (Chart 1).

2. Chemistry

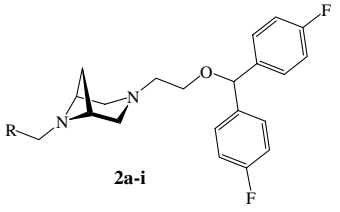
The starting material available for the preparation of compounds **2a**–**i** in Chart 1, that is the 6-*tert*-butyloxycarbonyl-3,6-diazabicyclo[3.1.1]heptane (**3**, Scheme 1), was synthesized as reported by us before.[10]

The N_3 atom in the diazabicyclo[3.1.1]heptane nucleus of compound **3** was alkylated by reacting with 2-[bis-(4-fluorophenyl)methoxy]ethyl iodide (**4**)[7] in the presence of a base to give compound **5** in good yield (see Scheme 1).

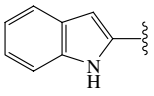
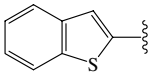
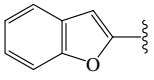
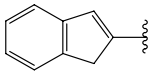
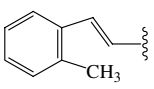
Deprotection of the carbamic nitrogen atom of **5** by removal of its N_6-Boc group, under mild acidic conditions, produced the desired 3-{2-[bis-(4-fluorophenyl)methoxy]ethyl}-3,6-diazabicyclo[3.1.1]heptane (**6**).



1



2a-i

2	R
a	
b	
c	
d	
e	

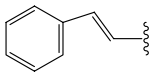
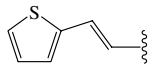
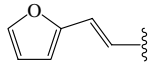
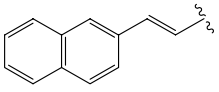
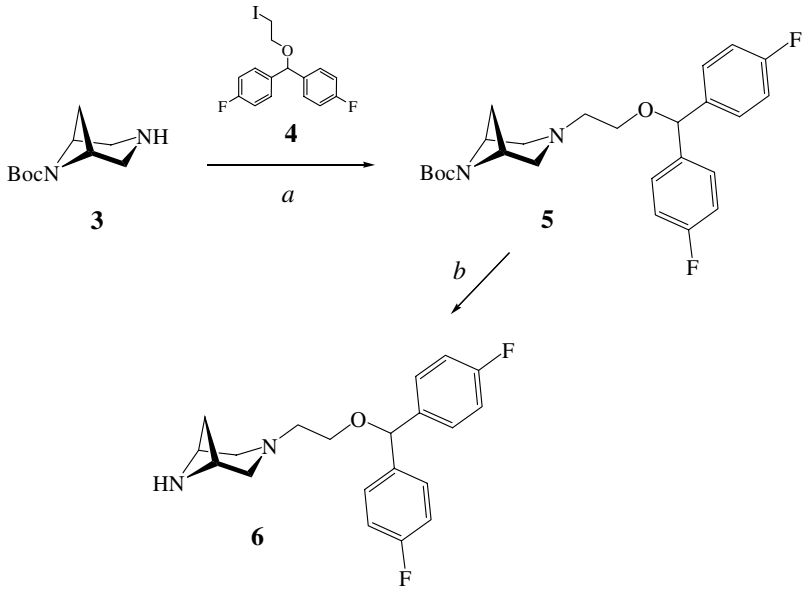
2	R
f	
g	
h	
i	

Chart 1. Molecular structures of the lead **1** and the designed compounds **2a**–**i**.



Scheme 1. Reagents and conditions: (a) K_2CO_3, 4-methylpentan-2-one, 115 °C, 17 h; (b) HCO_2H, room temperature, 4.5 h.

The availability of key intermediate **6** allowed the synthesis of the designed compounds **2a**–**i**.

In particular, the compounds **2a**–**c**, **g**, **h** were synthesized by a two-step process starting from **6** as shown in Scheme 2. The reaction at room temperature between the diamine **6** and the appropriate carboxylic acids commercially available **7a**–**c** and **9g**, **h**, in the presence of an drying agent as *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide (EDCI), allowed us to obtain the amides **8a**–**c** and **10g**, **h**, respectively. Thus, the intermediate amides underwent metal hydride reduction to afford the target amines **2a**–**c**, **g**, **h**.

Finally, the remaining designed compounds **2d**–**f**, **i** were synthesized by direct alkylation of **6** with the appropriate aldehydes **11d**,[11] **11e**,[12] **11f**,[e] and **11i**,[13] under reductive alkylation conditions with sodium cyanoborohydride and a catalytic amount of acetic acid (Scheme 2).

All compounds were converted into HCl or fumarate salts for biological evaluation.

3. Results and discussion

The ability to inhibit reuptake of [^{3}H]DA into striatal nerve ending (synaptosomes) of novel ligands **2** prepared in this study was determined using protocols described previously.[14] The reuptake values are listed in Table 1. For comparison purposes, data for lead compound **1**, expressed as IC_{50} value, and cocaine are also reported using those extrapolated from previous papers.[7,14]

As shown in Table 1, in general, the majority of compounds show very high potency in inhibiting DA uptake in comparison with lead compound **1**.

The highest activity is shown by the ligand **2a** having the indol-2-yl-methyl unit linked to N_6 of the N_3-bis-(4-fluorophenyl)-methoxyethyl-3,6-diazabicyclo[3.1.1]heptane scaffold, with a K_i of 5.5 nM, more potent than compound **1**. Compound **2a** serves as a convenient benchmark for all of the others in terms of presenting structure–activity relationship. Compounds **2b**–**d** have bioisosteric bicyclic units such as benzothiophene (**2b**), benzofurane (**2c**), or indene (**2d**) in place of the indole nucleus. These compounds, with the exception of **2d**, displayed a reduced activity, with K_i values of 35 and 37.5 nM, respectively, suggesting that the nature of the bicyclic unit in these ligands has interesting but unpredictable effects on dopamine reuptake activity.

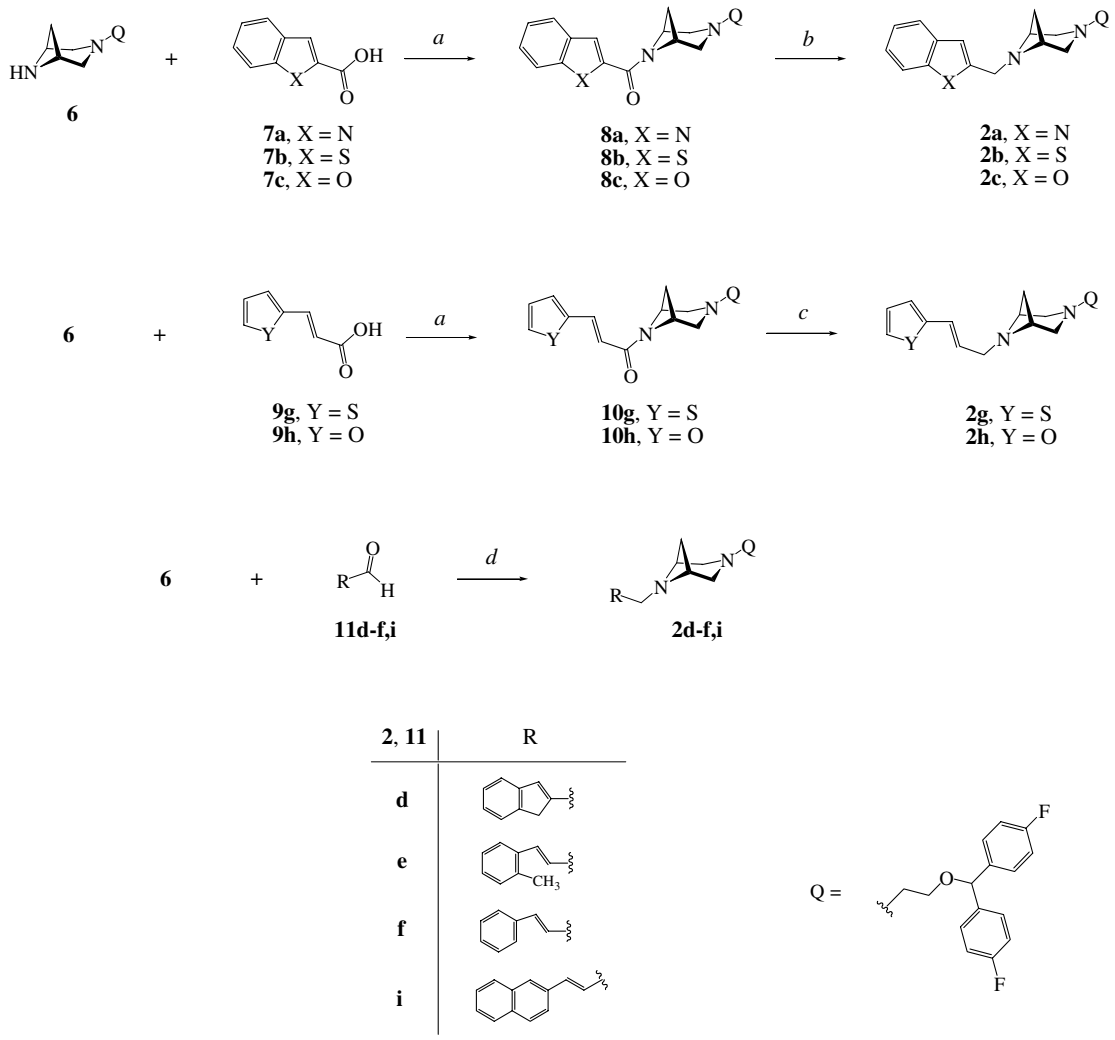
Compound **2e**, which contains a *o*-methylstiryl moiety obtained simply disconnecting the C_1–C_2 bond of the indenyl unit, maintained an activity that is comparable to that of **2a**.

Elimination of the methyl group of **2e** gave compound **2f** which showed a slight reduction of activity with respect to the ground term (**2f** vs **2a**).

Replacement of the phenyl group of **2f** with a thiophene (**2g**) or furane (**2h**) ring resulted in slightly reduced activity (**2g** and **2h** vs **2a**). Naphthalene derivative (**2i**) exhibited only a modest activity (K_i = 45.6 nM).

On the basis of the SAR that has emerged, we conclude that the N_3-difluoro-benzhydryloxyethyl-3,6-diazabicyclo[3.1.1]heptane play an important role in biological activity and could be viewed as a novel chemical template in DAT inhibitor design.

In this new series, moreover, five compounds, **2a**, **2d**, **2e**, **2f**, and **2h**, were the most potent derivatives with K_i values ranging from 5.5 to 9.1 nM.



Scheme 2. Reagents and conditions: (a) EDCI, CH_2Cl_2, room temperature, 20 h; (b) LAH, THF, room temperature, 14 h; (c) DIBALH, THF, 67 °C, 2 h; (d) $NaCNBH_3$, CH_3OH, CH_3COOH, room temperature, 20 h.

Finally, we believe that the knowledge gained from this study will facilitate the design of new candidates for safe and effective treatment of cocaine abuse.

Encouraged by these results we will continue to focus our efforts in further exploration of this novel class of DAT inhibitors.

4. Experimental

4.1. Chemistry

General informations. Melting points were obtained on an Electrothermal IA 9100 digital melting point apparatus or on a Kopfler melting point apparatus and are uncorrected.

Thin layer chromatography (TLC) was performed with Polygram® SIL N-HR/HV_{254} precoated plastic sheets (0.2 mm).

Flash chromatography (FC) was performed using Merck silica gel 60 (230–400 mesh ASTM).

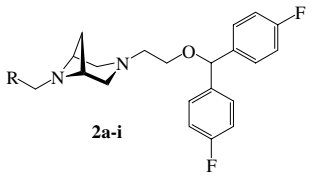
IR spectra were recorded as thin films (for oils) or Nujol mulls (for solids) on NaCl plates with a Jasco FT/IR 460 plus spectrophotometer and are expressed in ν (cm^{-1}).

All NMR spectra were taken on a Varian XL-200 NMR spectrometer with ^1H and ^{13}C being observed at 200 and 50 MHz, respectively. Chemical shifts for ^1H and spectra were reported in δ or ppm downfield from TMS [$(CH_3)_4Si$]. Multiplicities are recorded as s (singlet), br s (broad singlet), d (doublet), t (triplet), dd (double doublet), dt (double triplet), q (quartet), m (multiplet).

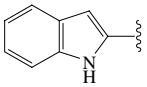
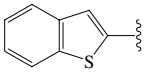
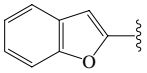
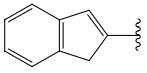
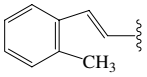
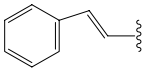
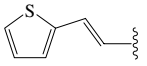
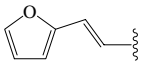
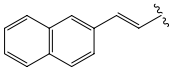
Elemental analyses were performed by Laboratorio di Microanalisi, Dipartimento di Chimica, Università di Sassari, Italy, and are within ±0.4% of the calculated values.

All reactions involving air- or moisture-sensitive compounds were performed under argon atmosphere.

Table 1. Inhibition of reuptake at DAT, K_i[a] (nM)



2a-i

Compound[b]	R	K_i uptake (nM)
2a		5.5 ± 0.9
2b		35 ± 5.7
2c		37.5 ± 5.9
2d		6.5 ± 1.2
2e		6.25 ± 1.1
2f		9.1 ± 1.2
2g		12.6 ± 1.9
2h		8.8 ± 1.6
2i		45.6 ± 2.3
1		40 ± 7[c]
(−)-Cocaine		111 ± 5

[a] Results are means ± SEM for three independent experiments.
[b] The inhibition of reuptake experiments of all compounds were carried out on their hydrochloride (**2a**) or fumarate (**2b–i**) salts.
[c] Expressed as IC_{50} value.

All final compounds were converted into the HCl or $HO_2CCH{=}CHCO_2H$ salts.

The general procedure for conversion to an HCl salt was the addition of excess ethereal HCl solution to a solution of the compound in ethanol or diethyl ether. The solvent was evaporated and the resulting salt was triturated with anhydrous ether and dried under vacuum.

The general procedure for conversion to a fumarate salt was the addition of a stoichiometric amount of a solution of fumaric acid in dry methanol to a solution of the compound in dry methanol. The solvent was evaporated and the resulting salt was triturated with anhydrous ether and dried under vacuum.

Unless otherwise specified, all materials, solvents, reagents, and precursors **7a–c**, **9g**, **h**, and **11f** were obtained from commercial suppliers.

4.2. 3-{2-[Bis-(4-fluorophenyl)methoxy]ethyl}-6-*tert*-butyloxycarbonyl-3,6-diazabicyclo[3.1.1] heptane (5)

A mixture of **3**[10] (1.30 g, 6.56 mmol), 2-[bis-(4-fluorophenyl)methoxy]ethyl iodide (**4**)[7] (3.09 g, 8.26 mmol), and K_2CO_3 (1.81 g, 13.12 mmol) in 4-methylpentan-2-one (55 mL) was stirred at reflux for 17 h. The solvent was then evaporated, the residue was taken up in $CHCl_3$, and the inorganic salt was filtered off. The filtrate was concentrated and the residue was purified by FC eluting with petroleum ether/EtOAc 7:3, to afford 1.96 g (67%) of **5** as yellow oil: R_f 0.29 (petroleum ether/EtOAc 7:3); bp 172 °C/0.1 mmHg; IR: 1600, 1710; ^{1}H NMR ($CDCl_3$) δ 1.42 (s, 9H), 1.63 (d, 1H, J = 7.4 Hz), 2.30–2.43 (m, 1H), 2.79–2.90 (m, 4H), 3.00–3.23 (m, 2H), 3.53 (t, 2H, J = 6.0 Hz), 4.00–4.09 (m, 2H), 5.32 (s, 1H), 6.96–7.05 (m, 4H), 7.22–7.35 (m, 4H). Anal ($C_{25}H_{30}F_2N_2O_3$) C, H, N.

4.3. 3-{2-[Bis-(4-fluorophenyl)methoxy]ethyl}-3,6-diazabicyclo[3.1.1]heptane (6)

A solution of **5** (0.65 g, 1.46 mmol) in 95% formic acid (25 mL) was stirred at room temperature for 4.5 h. The mixture was made alkaline with a 10% aqueous solution of NaOH and extracted with Et_2O. The organic layers were dried over Na_2SO_4 and concentrated to afford 0.28 g (56%) of **6** as yellow oil: R_f 0.27 ($CHCl_3$:MeOH 9:1); bp 198 °C/0.1 mmHg; IR: 1610, 3250; ^{1}H NMR ($CDCl_3$) δ 1.85 (d, 1H, J = 7.8 Hz), 2.40–2.55 (m, 1H), 2.72 (d, 2H, J = 10.6 Hz), 2.86 (t, 2H, J = 5.6 Hz), 3.13 (d, 2H, J = 10.8 Hz), 3.55–3.63 (m, 4H), 5.35 (s, 1H), 6.95–7.08 (m, 4H), 7.22–7.35 (m, 4H). Anal ($C_{20}H_{22}F_2N_2O$) C, H, N.

4.4. General procedures for the preparation of 8a–c and 10g, h

A solution of the appropriate carboxylic acid **7a–c**, **9g**, **h** (0.73 mmol) and *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide (EDCI) (0.17 g, 0.87 mmol) in CH_2Cl_2 (10 ml) was allowed to stir at room temperature for 5 min before a solution of **6** (0.20 g, 0.58 mmol) in CH_2Cl_2 (9 ml) was added. The reaction mixture was stirred at room temperature for 20 h, then washed with saturated aqueous $NaHCO_3$, H_2O, and brine. The organic layers were dried over Na_2SO_4 and concentrated. The crude product was purified by FC to afford the compounds **8a–c** and **10g**, **h**.

4.4.1. (3-{2-[Bis-(4-fluorophenyl)methoxy]ethyl}-3,6-diazabicyclo[3.1.1]heptan-6-yl)(1H-indol-2-yl)methanone (8a). Purified by FC (eluent: Et_2O); yield 85%; R_f 0.42

(Et_2O); mp 136–138 °C (Et_2O); IR: 1610, 1640, 3300; ^1H NMR ($CDCl_3$) δ 2.09 (d, 1H, J = 8.2 Hz), 2.60–2.72 (m, 1H), 2.77–2.87 (m, 2H), 2.96–3.36 (m, 4H), 3.45–3.55 (m, 2H), 4.55–4.67 (m, 1H), 4.77–4.87 (m, 1H), 5.25 (s, 1H), 6.71 (d, 1H, J = 1.2 Hz), 6.85–7.00 (m, 4H), 7.10–7.43 (m, 7H), 7.65 (d, 1H, J = 8.0 Hz), 9.17 (br s, 1H); ^{13}C NMR ($CDCl_3$) δ 28.97, 52.28, 54.25, 55.15, 59.95, 63.91, 66.73, 82.53, 104.33, 111.82, 115.03, 115.45, 120.60, 121.97, 124.61, 128.01, 128.37, 128.53, 129.83, 135.35, 137.75, 137.82, 159.64, 161.72, 164.53. Anal ($C_{29}H_{27}F_2N_3O_2$) C, H, N.

4.4.2. (Benzo[*b*]thiophen-2-yl)(3-{2-[bis-(4-fluorophenyl) methoxy]ethyl}-3,6-diazabicyclo [3.1.1]heptan-6-yl)methanone (8b). Purified by FC (eluent: Et_2O); yield 51%; R_f 0.29 (Et_2O); IR: 1600, 1650; ^1H NMR ($CDCl_3$) δ2.06 (d, 1H, J = 8.0 Hz), 2.58–2.68 (m, 1H), 2.80–2.92 (m, 2H), 2.95–3.18 (m, 3H), 3.30–3.58 (m, 3H), 4.53–4.62 (m, 1H), 4.75–4.86 (m, 1H), 5.27 (s, 1H), 6.85–7.00 (m, 4H), 7.15–7.26 (m, 4H), 7.36–7.44 (m, 2H), 7.72 (s, 1H), 7.78–7.89 (m, 2H). Anal ($C_{29}H_{26}F_2N_2O_2S$) C, H, N.

4.4.3. (Benzofuran-2-yl)(3-{2-[bis-(4-fluorophenyl)methoxy]ethyl}-3,6-diazabicyclo[3.1.1]heptane-6-yl)methanone (8c). Purified by FC (eluent: Et_2O); yield 60%; R_f 0.49 (Et_2O); IR: 1620, 1640; ^1H NMR ($CDCl_3$) δ 2.12 (d, 1H, J = 7.8 Hz), 2.58–2.70 (m, 1H), 2.80–2.90 (m, 2H), 3.00–3.40 (m, 4H), 3.42–3.60 (m, 2H), 4.55–4.65 (m, 1H), 5.02–5.12 (m, 1H), 5.27 (s, 1H), 6.85–7.00 (m, 4H), 7.15–7.55 (m, 8H), 7.66 (d, 1H, J = 7.6 Hz). Anal ($C_{29}H_{26}F_2N_2O_3$) C, H, N.

4.4.4. (*E*)-1-(3-{2-[Bis-(4-fluorophenyl)methoxy]ethyl}-3,6-diazabicyclo[3.1.1]heptan-6-yl)-3-(thiophen-2-yl)prop-2-en-1-one (10g). Purified by FC (eluent: EtOAc/Et_2O 6:4); yield 54%; R_f 0.23 (EtOAc/Et_2O 1:1); IR: 1600, 1650; ^1H NMR ($CDCl_3$) δ2.08 (d, 1H, J = 8.0 Hz), 2.42–2.58 (m, 1H), 2.80–2.88 (m, 2H), 2.90–3.28 (m, 4H), 3.49–3.54 (m, 2H), 4.40–4.50 (m, 2H), 5.29 (s, 1H), 6.22 (d, 1H, J = 15.2 Hz), 6.90–7.06 (m, 4H), 7.15–7.37 (m, 7H), 7.75 (d, 1H, J = 15.4 Hz). Anal ($C_{27}H_{26}F_2N_2O_2S$) C, H, N.

4.4.5. (*E*)-1-(3-{2-[Bis-(4-fluorophenyl)methoxy]ethyl}-3,6-diazabicyclo[3.1.1]heptan-6-yl)-3-(furan-2-yl)prop-2-en-1-one (10h). Purified by FC (eluent: EtOAc/Et_2O 3.5:6.5); yield 44%; R_f 0.29 (EtOAc/Et_2O 3.5:6.5); IR: 1610, 1660; ^1H NMR ($CDCl_3$) δ1.99 (d, 1H, J = 8.0 Hz), 2.35–2.46 (m, 1H), 2.76 (t, 2H, J = 5.4 Hz), 2.93 (d, 2H, J = 10.8 Hz), 3.16 (d, 2H, J = 11.0 Hz), 3.44 (t, 2H, J = 5.4 Hz), 4.37 (d, 2H, J = 5.8 Hz), 5.21 (s, 1H), 6.25 (d, 1H, J = 15.4 Hz), 6.41 (dd, 1H, J = 15.4 and 3.2 Hz), 6.80–6.98 (m, 4H), 7.08–7.20 (m, 6H), 7.36 (s, 1H). Anal ($C_{27}H_{26}F_2N_2O_3$) C, H, N.

4.5. General procedures for the preparation of 2a–c

To a suspension of lithium aluminum hydride (0.37 g, 9.84 mmol) in dry THF (40 mL) at 0 °C under a nitrogen atmosphere was added a solution of **8a**–**c** (2.46 mmol) in dry THF (40 mL). The mixture was allowed to slowly reach room temperature and was then stirred overnight. The reaction mixture was cooled to 0 °C, diethyl ether (40 mL) was added, and the reaction quenched with H_2O (0.37 mL), 2 M aqueous NaOH (0.37 mL), and H_2O (1.11 mL). The mixture was filtered and evaporated to give pure **2a**–**c**.

4.5.1. 3-{2-[Bis-(4-fluorophenyl)methoxy]ethyl}-6-[(1*H*-indol-2-yl)methyl]-3,6-diazabicyclo [3.1.1]heptane (2a). Yield 86%; R_f 0.51 ($CHCl_3$/MeOH 9.6:0.4); mp 108–110 °C (as dihydrochloride); IR: 1600, 3320; ^1H NMR ($CDCl_3$) δ 1.94 (d, 1H, J = 7.8 Hz), 2.40–2.50 (m, 1H), 2.88 (d, 2H, J = 10.8 Hz), 2.97 (t, 2H, J = 5.8 Hz), 3.13 (d, 2H, J = 11.4 Hz), 3.45–3.55 (m, 2H), 3.60 (t, 2H, J = 5.8 Hz), 3.77 (s, 2H), 5.37 (s, 1H), 6.23 (s, 1H), 6.95–7.17 (m, 6H), 7.22–7.38 (m, 5H), 7.52 (d, 1H, J = 7.6 Hz), 8.72 (br s, 1H); ^{13}C NMR ($CDCl_3$) δ (as dihydrochloride) 34.99, 42.26, 51.36, 53.77, 56.72, 60.08, 66.65, 82.54, 105.23, 111.88, 115.09, 115.52, 120.04, 120.44, 123.03, 126.77, 127.00, 128.39, 128.55, 137.09, 137.36, 141.47, 159.60, 164.49. Anal ($C_{29}H_{29}F_2N_3O$) C, H, N.

4.5.2. 6-[(Benzo[*b*]thiophen-2-yl)methyl]-3-{2-[bis-(4-fluorophenyl)methoxy]ethyl}-3,6-diaza bicyclo[3.1.1]heptane (2b). Yield 81%; R_f 0.21 (Et_2O); mp 116–118 °C (as difumarate); IR: 1603; ^1H NMR ($CDCl_3$) δ 1.84 (d, 1H, J = 7.8 Hz), 2.32–2.48 (m, 1H), 2.75–3.08 (m, 4H), 3.04 (d, 2H, J = 11.2 Hz), 3.45–3.60 (m, 4H), 3.77 (s, 2H), 5.28 (s, 1H), 5.66 (s, 1H), 6.84–7.00 (m, 4H), 7.10–7.33 (m, 6H), 7.52–7.73 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 28.47, 30.23, 44.88, 48.55, 55.03, 59.50, 66.95, 74.49, 82.51, 115.01, 115.43, 120.94, 122.16, 122.89, 123.61, 123.97, 126.37, 126.81, 127.95, 128.11, 128.38, 128.54, 137.88, 139.60, 143.14, 159.60, 164.49. Anal ($C_{29}H_{28}F_2N_2OS$) C, H, N.

4.5.3. 6-[(Benzofuran-2-yl)methyl]-3-{2-[bis-(4-fluorophenyl)methoxy]ethyl}-3,6-diazabicyclo [3.1.1]heptane (2c). Yield 62%; R_f 0.63 ($CHCl_3$/MeOH 9:1); mp 104–106 °C (as difumarate); IR: 1610; ^1H NMR ($CDCl_3$) δ 1.91 (d, 1H, J = 8.4 Hz), 2.45–2.60 (m, 1H), 2.85–3.02 (m, 4H), 3.19 (d, 2H, J = 10.8 Hz), 3.57–3.70 (m, 4H), 3.74 (s, 2H), 5.37 (s, 1H), 6.47 (s, 1H), 6.95–7.08 (m, 4H), 7.17–7.52 (m, 8H); ^{13}C NMR ($CDCl_3$) δ (as difumarate) 28.54, 31.20, 41.83, 47.68, 56.13, 60.36, 65.43, 67.35, 81.27, 113.98, 114.41, 119.94, 121.91, 123.10, 123.40, 127.40, 127.56, 131.03, 133.42, 135.38, 136.85, 153.41, 158.42, 163.30. Anal ($C_{29}H_{28}F_2N_2O_2$) C, H, N.

4.6. General procedures for the preparation of 2g, h

The appropriate amide **10g**, **h** (0.75 mmol) in dry THF (9 mL) was treated under argon with 1.5 M DIBALH (2.02 mL, 3.03 mmol) in toluene at room temperature. The mixture was stirred at reflux for 2 h, then cooled at room temperature, quenched by dropwise addition of H_2O (60 mL), and extracted with $CHCl_3$ (3× 30 mL). The organic layers were separated, dried over Na_2SO_4, and concentrated. The crude product was purified by FC to afford the amines **2g**, **h**.

4.6.1. 3-{2-[Bis-(4-fluorophenyl)methoxy]ethyl}-6-[(*E*)-3-thiophen-2-ylallyl]-3,6-diazabicyclo[3.1.1]heptane (2g). Purified by FC (eluent: $CHCl_3$/MeOH 9:1); yield 54%; R_f 0.51

($CHCl_3$: MeOH 9.2:0.8); mp 109–111 °C (as difumarate); IR: 1605; ^{1}H NMR ($CDCl_3$) δ 1.95 (d, 1H, J = 7.4 Hz), 2.45–2.63 (m, 1H), 2.90–3.00 (m, 4H), 3.06 (d, 2H, J = 11.0 Hz), 3.10–3.22 (m, 2H), 3.53–3.68 (m, 4H), 5.36 (s, 1H), 6.00 (dt, 1H, J = 15.4 and 6.4 Hz), 6.62 (d, 1H, J = 15.4 Hz), 6.90–7.38 (m, 11H); ^{13}C NMR ($CDCl_3$) δ 28.43, 47.21, 48.73, 54.94, 60.02, 67.05, 82.56, 115.09, 115.52, 124.19, 125.36, 127.27, 128.43, 128.59, 137.83, 159.69, 164.58. Anal ($C_{27}H_{28}F_2N_2OS$) C, H, N.

4.6.2. 3-{2-[Bis-(4-fluorophenyl)methoxy]ethyl}-6-[(*E*)-3-furan-2-ylallyl]-3,6-diazabicyclo [3.1.1]heptane (2h). Purified by FC (eluent: $CHCl_3$/MeOH 9.2:0.8); yield 43%; R_f 0.31 ($CHCl_3$/MeOH 9.2:0.8); mp 110–112 °C (as difumarate); IR: 1600; ^{1}H NMR ($CDCl_3$) δ 1.91 (d, 1H, J = 8.4 Hz), 2.52–2.65 (m, 1H), 2.90–3.02 (m, 4H), 3.15 (d, 2H, J = 11.4 Hz), 3.25–3.35 (m, 2H), 3.59 (t, 2H, J = 5.8 Hz), 3.70 (d, 2H, J = 6.0 Hz), 5.36 (s, 1H), 6.17 (dt, 1H, J = 14.0 and 5.2 Hz), 6.37 (d, 1H, J = 14.0 Hz), 6.95–7.07 (m, 4H), 7.22–7.36 (m, 7H); ^{13}C NMR ($CDCl_3$) δ 28.62, 46.80, 48.59, 55.05, 59.64, 67.07, 82.55, 107.19, 111.15, 115.08, 115.50, 120.46, 124.40, 128.44, 128.60, 130.86, 137.87, 141.74, 159.69, 164.57. Anal ($C_{27}H_{28}F_2N_2O_2$) C, H, N.

4.7. General procedures for the preparation of 2d–f, i

A solution of **6** (0.20 g, 0.58 mmol) and the required aldehydes **11d**,[11] **11e**,[12] **11f**, and **11i**[13] (0.70 mmol) in MeOH (9 mL) was treated with few drops of acetic acid, followed by $NaCNBH_3$ (51 mg, 0.81 mmol). The solution was stirred at room temperature for 20 h, and then the solvent was evaporated. The residue was dissolved in 5 mL of 1 N NH_4OH solution and extracted with diethyl ether. The organic layer was dried over Na_2SO_4, evaporated, and the residue was purified by FC to afford the amines **2d**–**f**, **i**.

4.7.1. 3-{2-[Bis-(4-fluorophenyl)methoxy]ethyl}-6-[(1*H*-inden-2-yl)methyl]-3,6-diazabicyclo [3.1.1]heptane (2d). Purified by FC (eluent: $CHCl_3$/MeOH 9.5:0.5); yield 46%; R_f 0.47 ($CHCl_3$/MeOH 9.5:0.5); mp 99–101 °C (as difumarate); IR: 1604; ^{1}H NMR ($CDCl_3$) δ 2.03 (d, 1H, J = 5.4 Hz), 2.55–2.70 (m, 1H), 2.90–3.08 (m, 4H), 3.12–3.28 (m, 2H), 3.41 (s, 2H), 3.55–3.68 (m, 4H), 3.70–3.77 (m, 2H), 5.37 (s, 1H), 6.68 (s, 1H), 6.94–7.08 (m, 4H), 7.10–7.43 (m, 8H); ^{13}C NMR ($CDCl_3$) δ 28.18, 40.55, 48.68, 54.86, 60.33, 67.02, 82.59, 115.11, 115.54, 120.49, 123.64, 124.42, 126.31, 128.43, 128.59, 129.70, 137.81, 141.53, 143.35, 144.49, 159.70, 164.59. Anal ($C_{30}H_{30}F_2N_2O$) C, H, N.

4.7.2. 3-{2-[Bis-(4-fluorophenyl)methoxy]ethyl}-6-[3-(2-methylphenyl)prop-2-enyl]-3,6-diazabicyclo[3.1.1]heptane (2e). Purified by FC (eluent: $CHCl_3$/MeOH 9.5:0.5); yield 62%; R_f 0.36 ($CHCl_3$/MeOH 9.5:0.5); mp 104–107 °C (as difumarate); IR: 1609; ^{1}H NMR ($CDCl_3$) δ 2.05–2.20 (m, 1H), 2.32 (s, 3H), 2.67–2.82 (m, 1H), 2.98–3.14 (m, 4H), 3.22–3.38 (m, 2H), 3.40–3.55 (m, 2H), 3.61 (t, 2H, J = 5.6 Hz), 3.80–3.88 (m, 2H), 5.37 (s, 1H), 6.03–6.22 (m, 1H), 6.82 (d, 1H, J = 16.2 Hz), 6.95–7.05 (m, 4H), 7.10–7.47 (m, 8H); ^{13}C NMR ($CDCl_3$) δ 19.81, 27.70, 48.98, 54.62, 60.93, 66.99, 82.56, 115.09, 115.52, 123.64, 125.85, 126.16, 127.84, 128.40, 128.55, 130.24, 132.38, 135.27, 137.72, 137.77, 159.67, 164.56. Anal ($C_{30}H_{32}F_2N_2O$) C, H, N.

4.7.3. 3-{2-[Bis-(4-fluorophenyl)methoxy]ethyl}-6-cinnamyl-3,6-diazabicyclo[3.1.1]heptane (2f). Purified by FC (eluent: $CHCl_3$/MeOH 9.5:0.5); yield 45%; R_f 0.35 ($CHCl_3$/MeOH 9.5:0.5); mp 123–125 °C (as difumarate); IR: 1610; ^{1}H NMR ($CDCl_3$) δ 2.01 (d, 1H, J = 7.8 Hz), 2.50–2.62 (m, 1H), 2.90–3.00 (m, 4H), 3.15 (d, 2H, J = 11.2 Hz), 3.30 (d, 2H, J = 5.4 Hz), 3.60 (t, 2H, J = 5.6 Hz), 3.65–3.73 (m, 2H), 5.37 (s, 1H), 6.21 (dt, 1H, J = 16.0 and 5.4 Hz), 6.52 (d, 1H, J = 16.0 Hz), 6.95–7.08 (m, 4H), 7.20–7.38 (m, 9H); ^{13}C NMR ($CDCl_3$) δ 28.35, 48.66, 54.92, 59.95, 67.01, 82.50, 115.03, 115.45, 124.91, 126.22, 127.47, 128.39, 128.46, 128.55, 132.59, 136.70, 137.81, 137.87, 159.62, 164.51. Anal ($C_{29}H_{30}F_2N_2O$) C, H, N.

4.7.4. 3-{2-[Bis-(4-fluorophenyl)methoxy]ethyl}-6-[(*E*)-3-(naphthalen-2-yl)allyl]-3,6-diazabicyclo[3.1.1]heptane (2i). Purified by FC (eluent: $CHCl_3$/MeOH 9.6:0.4); yield 57%; R_f 0.34 ($CHCl_3$/MeOH 9.6:0.4); mp 116–118 °C (as difumarate); IR: 1605; ^{1}H NMR ($CDCl_3$) δ 2.01 (d, 1H, J = 7.4 Hz), 2.48–2.62 (m, 1H), 2.85–3.03 (m, 4H), 3.16 (d, 2H, J = 11.2 Hz), 3.33 (d, 2H, J = 5.6 Hz), 3.55–3.71 (m, 4H), 5.38 (s, 1H), 6.32 (dt, 1H, J = 16.2 and 5.6 Hz), 6.67 (d, 1H, J = 16.2 Hz), 6.95–7.07 (m, 4H), 7.22–7.36 (m, 4H), 7.38–7.45 (m, 2H), 7.57 (d, 1H, J = 8.8 Hz), 7.66 (s, 1H), 7.70–7.82 (m, 3H); ^{13}C NMR ($CDCl_3$) δ 28.59, 47.54, 48.75, 55.08, 59.86, 67.10, 82.57, 115.09, 115.52, 123.44, 125.76, 126.09, 126.21, 127.61, 127.90, 128.15, 128.46, 128.63, 132.32, 132.90, 133.54, 134.37, 137.89, 137.95, 159.70, 164.59. Anal ($C_{33}H_{32}F_2N_2O$) C, H, N.

4.8. Biology: materials and methods

4.8.1. Chemicals. [^{3}H]DA (specific activity 60 Ci/mmol) was obtained from New England Nuclear (Boston, MA, USA). For uptake experiments, drugs were dissolved in DMSO. DMSO concentration in the different assays never exceeded 0.1% (v/v) and was without effect on uptake.

4.8.2. Animals. Male Sprague–Dawley albino rats weighting 150–200 g (Charles River, Calco, LC, Italy) were housed three or four per cage and given free access to food and water under controlled conditions of temperature (22 ± 1 °C) and humidity (65 ± 5%) with a 12 h light/dark cycle. All experimental procedures were performed in strict accordance with the EC regulation for care and use of experimental animals (EEC N°86/609).

4.8.3. Tissue preparation. Rats were killed by decapitation and the brains were rapidly removed and placed on an ice-cold plate. The striatum was quickly dissected over ice and homogenized using a Teflon-glass homogenizer (clearance approximately 0.009 in.) with 10 up-and-down strokes at 850 rpm in 10 volumes (w/v) of ice-cold 0.32 M sucrose containing 10 mM glucose and

10 mM Tris (pH 7.4). The homogenate was then centrifuged at 1000*g* for 10 min at 4 °C and the resulting supernatant centrifuged at 12,000*g* for 20 min at 4 °C to obtain a crude synaptosomal fraction (P2). The synaptosomes were resuspended in the assay buffer immediately before use.

4.8.4. Assay of [^{3}H]DA uptake. The synaptosomes-containing pellet was resuspended in a modified Krebs–Henseleit buffer and assayed according to Schoemaker and Nickolson.[15] The standard uptake medium (pH 7.4 a 37 °C) contained 140 mM NaCl, 5 mM KCl, 1 mM $MgCl_2$, 10 mM glucose, 1.2 mM $CaCl_2$, 10 mM Hepes, 1 mM ascorbic acid, 0.054 mM EDTA, and 0.1 mM pargyline.

Triplicate aliquots (90–150 μg protein) of the P2 fraction were preincubated for 10 min at 37 °C in the above-mentioned buffer prior to the initiation of uptake. The uptake was started by adding 50 nM [^{3}H]DA. After incubation at 37 °C for 5 min the uptake was stopped by rapid filtration under vacuum through glass-fiber filter (Whatman GF/C) using a single filter holder (Millipore). Filters were then washed three times with 5 ml of ice-cold 0.9% NaCl solution. The total time for the three washes was about 5 s, after which the filters were removed immediately and placed into vials containing 10 ml of scintillation liquid (Ultima Gold MV, Packard, Meridien, USA) and the filter-bound radioactivity was measured in a liquid scintillation counter (Tricarb 2900, Packard, Meridien, USA).

The values of [^{3}H]DA uptake obtained from the synaptosomal fraction incubated over ice (blanks) were subtracted from the corresponding samples at 37 °C. Inhibition curves were plotted and analyzed by computer with appropriate software (Kell 6.0, Biosoft, Cambridge, UK; graph Software, San Diego, CA, USA). IC_{50} values were derived from the calculated curves and converted to K_i values as previously described.[16]

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